# SYNTHETIC ORGANIC CHEMICALS

## Experimental Methods in Research with Stable Isotopes

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THE APPLICATION of stable isotopes to chemical and biological problems depends upon the development of suitable apparatus and experimental technic, as well as upon the availability of tracer materials. Heretofore, only those laboratories that were equipped to separate stable isotopes have had access to them, with the exception of deuterium. Now, for the first time, $N^{15}$ concentrates are available commercially from the Eastman Kodak Company.

In any application where "tagged" atoms are employed, the investigation may be divided into three steps: first, the preparation of compounds containing the isotopic material; second, the isolation of the product material and its treatment for isotopic analysis; third, the measurement of the isotope ratio in the starting material and the final product.

### Preparation

In the first stage of the investigation, the tracer element must be incorporated in the compound that is to be studied. To be useful, the "tagged" atom must be bound to the remainder of the molecule by a stable linkage that is not subject to exchange reactions. It is well established (1) that hydrogen that is not directly

attached to carbon but rather to oxygen or nitrogen, as in OH, COOH, and NHR groups, is labile and exchanges readily with the hydrogen of water. Similarly, hydrogen attached to a carbon atom adjacent to a carbonyl group is exchangeable by enolization, which causes the hydrogen to be attached temporarily to oxygen. In the use of $N^{15}$ as an indicator of the amino group in amino acids, it has been shown (2) that exchange of nitrogen does not occur between the labeled amino acids and other components of the system. In the case of $C^{13}$, the exchange of carbon atoms that are linked in a $C-C$ arrangement is very unlikely.

The synthesis of the compound chosen must result in a high yield on the basis of the isotope employed, or at least permit a quantitative recovery of the unreacted tracer material. In typical situations already studied, amino acids have been prepared (3) by the methods of Knoop and Oesterlin and of Gabriel and Kroseberg. Deuterium-containing compounds are often made by the reduction of unsaturates with deuterium gas. In a few cases advantage has been taken of biological synthesis, as in the preparation of unsaturated deutero-fatty acids (4). Sodium cyanide will probably be the starting point for the synthesis of compounds containing $C^{13}$.

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### Isolation

After incorporating the tracer material in the compound to be studied, and carrying out the specific reaction, whether this be chemical or biological, the experimenter must isolate, purify, and treat the compound that has been produced for isotopic analysis. The sample that is isolated must contain no unreacted material, since this would have an abnormal isotope ratio; other products containing the tracer element must also be absent. Recovery of the compound to be analyzed need not be quantitative.

For the most convenient analysis, using the mass spectrometer, the isotopic element must be converted into a gaseous compound. Deuterium is thus analyzed in the form of deuterium gas. The compound suspected of containing deuterium is burned in a stream of oxygen. The water obtained is then electrolyzed, and the hydrogen-deuterium mixture collected for transfer to the mass spectrometer. The $C^{13}$ content of organic material may be determined most simply on $CO_2$, prepared by burning the compound in oxygen.

The analysis for $N^{15}$ is made on nitrogen gas. The nitrogen-containing compound is first subjected to a Kjeldahl digestion. The ammonium salt is then converted into molecular nitrogen by reaction with alkaline hypobromite, in a vacuum, to eliminate atmospheric nitrogen. The apparatus required for any of these conversions is not extensive, and the treatment of samples for analysis on a routine basis requires only a short time.

### Measurement

The apparatus required in the third phase of the investigation, the measurement of isotope ratios, is somewhat more elaborate. The content of deuterium in the water of combustion may be obtained, after careful purification, by accurate measurement of its density. A modification (5) of the "falling-drop" method is probably the most convenient, and is applicable to the small samples that are usually available. However, since the density method cannot be applied to the determination of tracers other than deuterium, its usefulness is limited. The mass spectrometer, on the other hand, is equally suitable for the analysis of deuterium, carbon, nitrogen, oxygen, sulfur, and other elements, and it is recommended for any program involving the use of different isotopes.

Prior to 1940, mass spectrometers were not available as commercial instruments, and those that had been described in the literature were not suitable for routine analyses in tracer studies. In that year, however, Nier (6) designed a mass spectrometer that is primarily intended for use in laboratories where tracer isotopes are being employed for investigation. This instrument is characterized by a simple magnet that is powered by two 6-volt storage batteries. In operation, the gas to be analyzed is admitted to the evacuated spectrometer tube, through a capillary leak, and is ionized by electrons emitted from a hot filament. The ions are accelerated between parallel plates that are kept at a potential difference of 500–1000 volts; they are collimated by parallel slits in these plates, and, finally, are allowed to pass between the poles of the magnet. In the magnetic field the beam of ions is dispersed to produce a mass spectrum. Thus, molecules of $N^{14}N^{14}$ are segregated from molecules of $N^{14}N^{15}$ and are collected separately. The relative amounts of the two species are determined by measurement of the ion currents with an electrometer-tube amplifier. A high-vacuum system that consists of diffusion and mechanical pumps completes the apparatus.

Recently, the production of a commercial model of a mass spectrometer was announced (7). The parts of this instrument are built to a high degree of precision and are interchangeable. Adjustments are readily accomplished, operation is simple, and the whole instrument is assembled for portable use.

In addition to its use in tracer studies, the mass spectrometer will often prove of value in applications not involving isotopes; for example, as a sensitive apparatus for gas analysis. Applications have included the analysis of furnace atmospheres, and reaction mixtures, where the presence of a given gas as a product, or intermediate, may be detected in extremely low concentration by the formation of ions of characteristic mass in the spectrometer. Another promising application seems to be in the analysis of mixtures of hydrocarbons, in which field considerable research on the use of the mass spectrometer is now in progress.

### Literature Cited

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# The Testing of Eastman Organic Chemicals

IN THE LISTS of *Eastman Organic Chemicals*, the majority of the names of chemicals are followed by some designation, such as melting point, MP, boiling point, BP, or the expression (Pract.) or (Techn.). The chemicals are referred to by numbers, some of which are preceded by T or P, representing Technical or Practical grades. These designations are indications of the quality of the chemicals. All compounds that come into the *Synthetic Organic Chemistry Department* of the Kodak Research Laboratories, whether they are manufactured in the Department or obtained outside, are subjected to proper analysis and approval before they are finally accepted for stock and listing in the catalog.

Not only are the fundamental constants checked, but, wherever possible, an analysis is made to determine purity. Physical constants are not always an absolute guarantee of purity. Such compounds as acids and anhydrides, aldehydes and ketones, esters and inorganic acid salts, and organometallic compounds are analyzed. The literature is being studied constantly for new methods and those that are likely to be of value are kept on file and checked whenever the occasion demands. Faster and more accurate methods are being sought continually, since several thousand different chemicals are tested annually.

*Melting points* are checked carefully. The range of melting and the clarity with which a compound melts are noted. When the melting point of a compound is in doubt, a comparison is made with a standard. If the compound fails to meet the requirements of the standard, it is rejected. Various methods are employed, all depending upon the range at which solidification and melting take place. Mixed melting points help to establish the identity of many compounds.

*Boiling points*, or more accurately, boiling-point ranges, are taken continually. Different types of columns have been tried, but it has been found that a modified Widmer column is most efficient. Distilling columns which are easy to clean and, above all, those that give the most efficient fractionation are used. A standard rate of distillation has been established, whether it be under normal pressure or vacuum.

To correlate the findings of the boiling points, *densities* have proved to be of great worth. Since literature values are not always recorded under the same conditions, the laboratory has found it advantageous to take readings at 20°C. First, the density is checked at literature-recorded temperatures; then at 20°C. The purity of organic halides, such as methyl and ethyl iodides, butyl bromide, and a number of others, is indicated

most quickly by density measurements.

*Refractive indices* are likewise taken at 20°C. The Abbé refractometer with constant temperature control is in regular use. For example, compounds such as α-bromonaphthalene are accepted only when their refractive index falls within certain narrow limits, as measured by means of the refractometer.

Boiling-point data, however, are not always sufficient to establish the identity of certain compounds, and it is necessary, therefore, to prepare known derivatives of them. In the manufacture of diethyl ketone, for instance, methyl-n-propyl ketone is sometimes the result. This compound has physical properties identical with those of diethyl ketone, and only the preparation of a dinitro-phenylhydrazone will distinguish between the two. In this way the identity is definitely established.

It has been found that potentiometric titrations and the determination of pH values are important. Whenever colored solutions are to be analyzed, the potentiometer has proved its worth.

Special equipment is used in the spectrometric determination of sulfonphthalein indicators. Amino acids, as well as sugars, are determined by rotation values on the polariscope. Microchemical analyses are made whenever necessary.

As a result of such insistence on the maintenance of standards of quality, and their description in all the catalog lists of chemicals, the chemist can rely on *Eastman Organic Chemicals*.

## An Antifoam Device

IN CERTAIN EVAPORATION OPERATIONS that are conducted under reduced pressure, it is natural for foaming to occur. This creates the problem of preventing the foam from contaminating the distillate. Furthermore, aqueous solutions of salts sometimes have a tendency to foam, and, as the solution becomes concentrated, the residual liquor may creep.



A device of the type illustrated at the left is helpful in such situations. A still-head is joined to a relatively large, long-necked flask by means of a large-diameter tube. Extending from the bottom of the flask is a trapped return tube. When expanding into the flask, the foam breaks; then it drains into the return tube and back into the lower end of the still-head.

The setup shown consists of a 22-liter distillation flask, a 12-liter receiver, and an antifoam unit of 3 liters' capacity. It has been employed successfully in concentrating aqueous solutions of *dl*-malic acid and aliphatic amine hydrochlorides.